methods of sample preparation on the overall objectives of detecting novel bioactive natural products in plant biomass.

Evaluating Natural Products for Potentially Therapeutic Biological Effects

The core foundation of every drug discovery program is the approach to the detection of desirable biological activities. The goal of biological evaluation is to identify compounds with selective and specific biological effects on contemporary and relevant disease targets, and to effectively predict in vivo efficacy, toxicity and pharmacokinetics. Typically, a tier of assays may be established (2), beginning with a primary assay that has a relatively high throughput capacity and is designed to detect samples with the most promise for yielding interesting compounds, followed by secondary assays to corroborate, quantify, and define specific activities, and tertiary assays designed to assess the clinical potential of promising compounds (usually animal models). The primary assay is usually designed to "screen" out the vast majority of samples with low to moderate activity or with non-selective activity. Prior to major advances in molecular biology, molecular pharmacology, and genomics, most assays for the discovery of new drugs were based on identifying compounds that effected an observable response in animals, or, for antibiotics, that inhibited the growth of the target pathogens. While this had the advantage of detecting those agents active in whole cell or whole animal systems, it was very laborious, expensive, and often impractical. With the advent of molecular biology techniques, primary assays are now typically mechanism-based, molecular target-specific bioassays. These are usually developed as high throughput screens, designed to screen large libraries of samples. It is important to recognize that specific challenges are associated with screening natural product extracts (as opposed to pure compounds or mixtures of compounds, such as might be produced through combinatorial synthesis). In particular, natural product extracts typically are colored, insoluble and consist of numerous compounds that may interact (synergistic and antagonistic), may result in false positives, and present significant challenges in assay sensitivity. Secondary assays are usually designed to corroborate and quantitate the activity observed in the primary assay, to establish the spectrum of activity, to provide insight into the mode of action, and to predict in vivo pharmaceutical properties. Important criteria for determining the relative importance of a lead compound usually include evidence to suggest it acts by a novel mechanism of action and evidence that it possesses desirable pharmaceutical properties. Claeson and Bohlin have provided an excellent overview of some of these challenges in designing bioassays specifically for natural products screening (2).

Key decisions in designing, utilizing and interpreting the results of biological assays include the selection of the target (therapeutic, systemic, cellular, molecular), establishing a threshold for "active," determining whether high throughput screening is necessary and appropriate for each assay, and, an important and consistently difficult problem, designing in vitro bioassays that will correlate with, and effectively predict in vivo outcomes. Advances in functional vs. affinity genomics and the use of transgenic systems, along with miniaturization and automation of biological assay techniques, have revolutionized the biological evaluation of all products, natural and synthetic. Additionally, the use of molecular biology techniques, such as incorporation of luciferase reporter genes into cell lines, and advances in instrumentation that allow the use of sensitive luminescent and colorimetric assays has provided a means of coupling bioassays with chromatographic and spectroscopic techniques, in some cases allowing on-line bioassay capability.

Identifying the Biologically Active Natural Product

One of the major challenges in natural product drug discovery is determining which of a number of approximately equally active samples to pursue for further study, usually for isolation and structure elucidation of the active constituent(s). This is best accomplished through a system of prioritization coupled with the process known as dereplication. Since isolation and structure elucidation may be among the most laborious, time-consuming, and expensive steps in natural product drug discovery, much attention is given to developing reliable methods of dereplication. The goal of dereplication is to select, for follow-up isolation and structure elucidation, only extracts that are likely to yield novel chemotypes, i.e., to "de-select" compounds with known activity profiles or structures. One of the best ways to achieve dereplication is to access unique sources, so that there is a greater assurance of obtaining novel chemotypes. Also, advances in coupling spectroscopic and chromatographic techniques can provide rapid and reliable structural information on small quantities of material, thus facilitating dereplication (20). Judicious use of the literature is a critical aspect of successful dereplication.

It is also important to prioritize samples for further study. Priority assignment may be based on a combination of factors, including biological activity profile, the results of literature searches that indicate minimal previous work on a specimen, and the availability of sufficient biomass for larger scale fractionation. Typically, once a priority position is assigned to a given sample, bioassay-directed fractionation is carried out to isolate pure active constituents. Recent developments that have affected the ability to isolate minor active constituents include the use of supercritical fluid chromatography, capillary electrophoresis, countercurrent chromatography, centrifugal partition chromatography, and spin columns, among others.

Structural characterization of isolated active compounds is accomplished most often using state-of-the-art